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Laser Action in Dye-Infused Biological Tissue

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Abstract

The narrowing of the spectral linewidth and the increasing of the peak intensity characteristic of laser action was observed in emission spectra of dye-infused biological tissues. The fresh tissue was infused with a solution of Rhodamine 640 perchlorate in ethanol and then excited with frequency-doubled Q-switched Nd:YAG laser pulses. The sharp spectral peaks of laser action in tissues may find applications in detection of superficial disease.

Key Words: Tissue Optics, Laser Action, Fluorescence, Amplification of Spontaneous Emission, Turbid Media.

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Introduction

Laser action in turbid media made of TiO_2 in methanol mixed with dye solution was first observed by Lawandy *et al.* recently, where the dye solution was Rhodamine 640 perchlorate dissolved in methanol.[1] The turbid media were excited with a pulsed laser, and a surprisingly low threshold excitation energy was required to generate the laser action. The mechanism for the laser action was speculated to be scattering-enhanced amplification of spontaneous emission.[2] The scattering property of the turbid media increased the path length of light in the gain medium, which consisted of the dye molecules excited by the pulsed laser. When the path length exceeded a critical gain length, spectral narrowing of the spontaneous emission linewidth occurred. It has been known that the spectral linewidth was inversely proportional to the square root of the light propagation length for both homogeneously and inhomogeneously broadened transitions under unsaturated-gain conditions.[3]

The above experiment was conducted in physical turbid media. Knowing that most biological tissues are intrinsically scattering, we hypothesized that the laser action could be generated in biological tissues and hoped that the sharp and strong spectral peak could result in high-sensitivity diagnostic techniques in medicine. This article presents our experimental findings on laser action in biological tissue. We have replaced the methanol with ethanol as the solvent of the dye because ethanol is less biologically toxic than methanol. Other investigators have also independently studied laser action in biological tissues.[4-6]

Methods and Materials

Dye solutions were prepared using Rhodamine 640 perchlorate (molecular weight, 591) mixed in a 70% solution of ethanol. The concentration of dye will be expressed in the units of M. Muscle tissue was freshly excised from killed Fisher 344 rats and then divided into multiple pieces weighing 540 ± 10 mg each. A total of 0.2 ml of dye solution was injected into various sites of each piece of tissue and allowed to diffuse evenly for 10 min. The actual absorbed amount was 0.09 ± 0.017 ml, which was measured by weighing the tissue before and 10 min. after the dye injection. The dye solution that leaked out of the tissue was excluded.

The tissue sample was placed on a plastic dish and covered with a thin piece of glass to prevent the tissue from drying (**Fig. 1**). Then the dish was placed on a height-adjustable platform so the spot area of the laser beam on the sample surface could be varied. A linearly polarized frequency-doubled (532 nm) Q-switched Nd:YAG laser pulse of 10-ns duration was repeated at a rate of 10 Hz. Two prisms routed the laser light, which was focused by a lens of

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10-cm focal length and normally incident to the sample surface. The pulse energy was monitored by splitting a portion of the laser beam into an energy detector connected to an oscilloscope. An optical fiber (core diameter, 600 μm; N.A., 0.44) of 45° incidence collected the emission spectra near the samples, where the distance between the tip of the optical fiber and the incident point of the excitation laser beam on the tissue surface was 1.5 cm. The polarization plane of the excitation laser light was perpendicular to the plane formed by the incident laser light and the collection optical fiber. An optical multichannel analyzer (OMA) system that was synchronized with the laser pulses analyzed the spectra that passed a 570-nm long-pass filter, a narrow slit, and a spectrograph. A computer averaged the spectra over five measurements and displayed the averaged spectra.

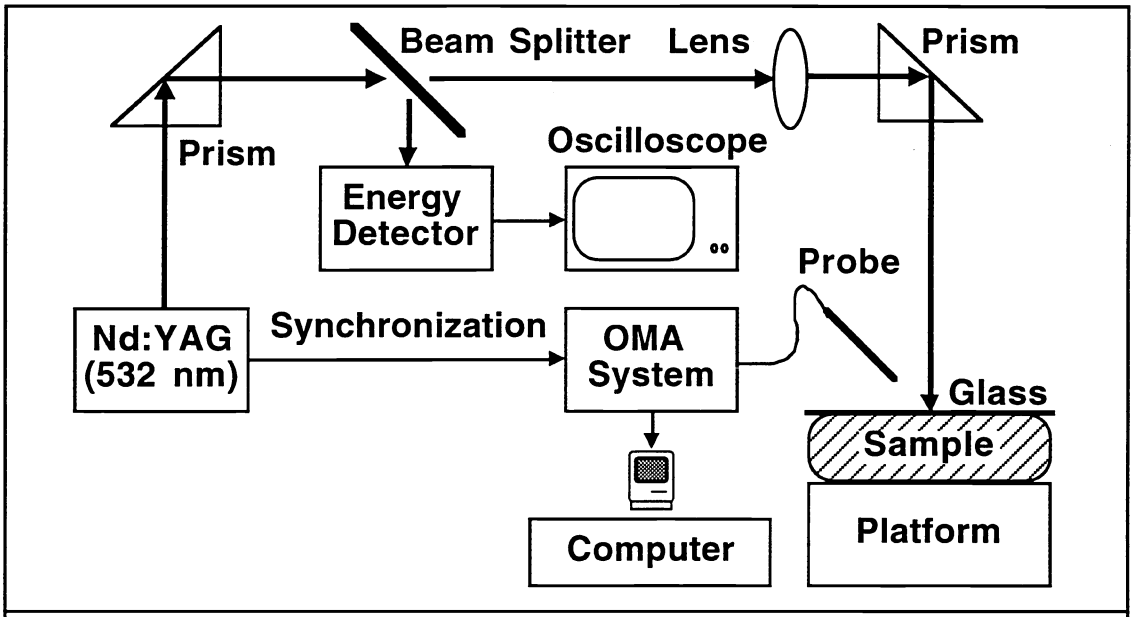

Fig. 1. Schematic of the experimental set-up. The thick and thin arrows represent optical and electronic signals, respectively. The thickness of the glass layer was 0.016 cm.

Results

Figure 2 compares the emission spectra of the dye-infused muscle tissues with respectively low and high excitation energy. The dye concentration in the ethanol solution was 1.69×10^{-3} M. The spot area of the laser beam on the sample surface was 1.47 mm^2. When the excitation energy of the laser beam on the dye-infused muscle tissue was increased from

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0.07 mJ to 3 mJ, the emission spectrum changed from fluorescence spectrum to laser-action spectrum, where the laser action had an ~19% full width at half maximum (11 nm versus 58 nm) and 52 times the peak height of the fluorescence spectrum.

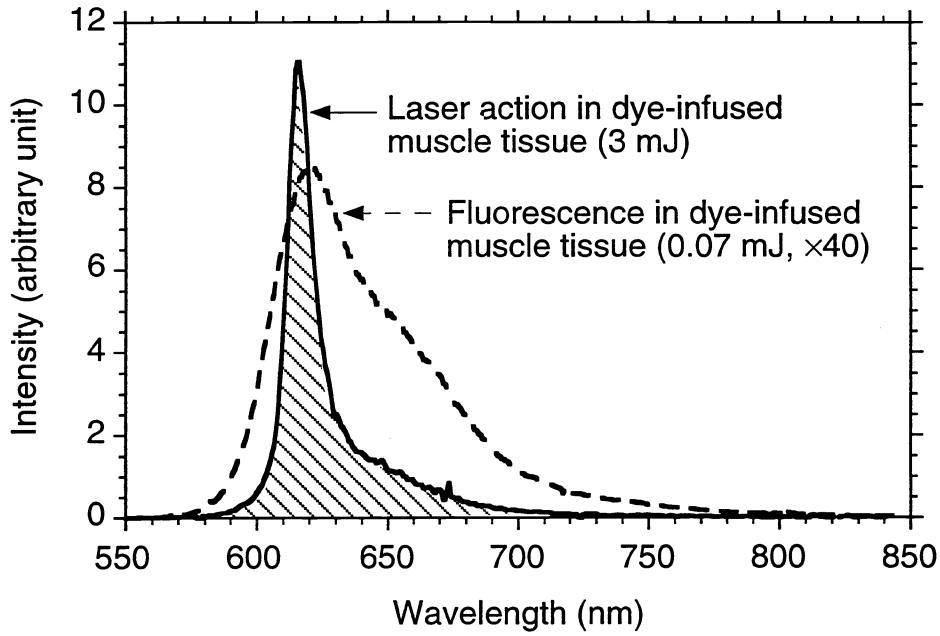

Fig. 2. Emission spectra of the Rhodamine 640 perchlorate dye-infused muscle tissue with 0.07 and 3 mJ of excitation-laser energy, respectively. The excitation-laser energy values and the scaling factors of the spectra are listed in parentheses.

Monte Carlo simulations[7,8] were used to compute the excitation light fluence distribution in the tissue used in the above experiments with Rhodamine 640 perchlorate. **Figure 3** shows the schematic of the setup that was used in the Monte Carlo simulation. **Figure 4** shows the internal distribution of excitation-light fluence in the tissue when the intrinsic absorption coefficient of the tissue was used and the absorption of the Rhodamine 640 perchlorate dye was neglected. The optical properties that were used for Fig. 4 at 532 nm wavelength were: absorption coefficient 1.0 cm^{-1}, reduced scattering coefficient 10 cm^{-1}, and assumed anisotropy factor 0.9, which were extrapolated values based on the data in the literature.[9]

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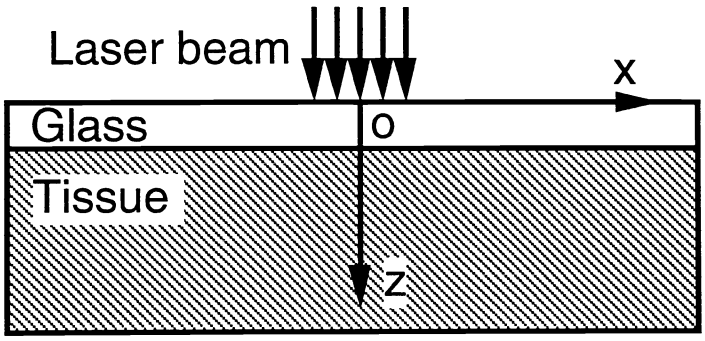

Fig. 3. Setup that was used for Monte Carlo simulations of excitation light distribution in Rhodamine 640 perchlorate dye-infused rat muscle. The thickness of the glass layer was 0.016 cm.

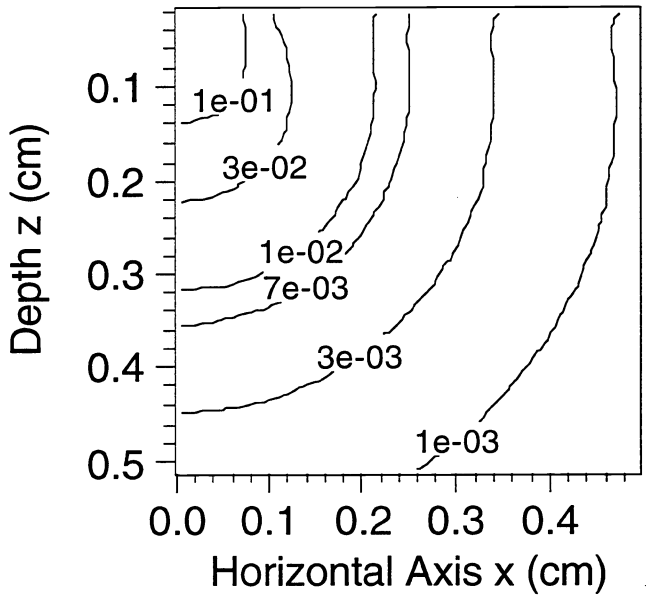

Fig. 4. Fluence (J/cm^2) of excitation light at 532 nm wavelength using the intrinsic absorption coefficient of the tissue system and neglecting the dye absorption. The optical properties of the tissue at 532 nm wavelength were: absorption coefficient 1.0 cm^{-1}, reduced scattering coefficient 10 cm^{-1}, and assumed anisotropy factor 0.9. The diameter of the laser beam was 0.137 cm (radius, 0.0685 cm), corresponding to the 1.47 mm^2 beam area. The pulse energy and pulse width were 3 mJ and 10 ns, respectively.

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Discussion and Conclusions

Figure 2 shows that laser action can be obtained in dye-infused biological turbid media similarly as in physical turbid media. The dye concentration was 1.69×10^{-3} M in the ethanol solution, and was reduced to 2.4×10^{-4} M in the tissue because of the additional tissue volume (see Methods and Materials). The extinction coefficient of Rhodamine 640 perchlorate at the excitation wavelength (532 nm) was ~3.2×10^4 M^{-1} cm^{-1}. The low-power absorption coefficient of the dye in the tissue was then $\ln(10) \times (2.4 \times 10^{-4}\ M) \times (3.2 \times 10^4\ M^{-1}\ cm^{-1}) = 18\ cm^{-1}$, which was much higher than the intrinsic absorption coefficient of the rat muscle (~1 cm^{-1}). The reduced scattering coefficient of the rat muscle at the excitation wavelength (532 nm) was approximately 10 cm^{-1}; and hence the transport mean free path length in the native tissue was 0.09 cm. At low excitation intensity, the light energy will be limited to a small zone. However, when the excitation intensity approaches the saturation intensity of the dye (0.7 MW/cm^2),[1] the saturated dye becomes transparent, and the excitation light energy will be distributed into a larger volume in the tissue.

Based on the pulse width of the excitation laser beam (10 ns), the saturation intensity of the dye (0.7 MW/cm^2) can be converted into saturation fluence 7.0×10^{-3} J/cm^2, of which the contour lines were bold in Fig. 4. The saturation region enclosed by the contour line of the saturation fluence provides a gain medium for amplification of spontaneous emission. The computation for Fig. 4 ignored the absorption of the dye solution and used the intrinsic absorption of the rat muscle; hence, the saturation region in Fig. 4 represents the maximal volume of the gain medium.

In a nonturbid Rhodamine 640 perchlorate dye solution, the saturation region would be a narrow cylinder. Therefore, the shape of the saturation region in the turbid media will facilitate the amplification of spontaneous emission because photons can travel longer in an approximately semi-spherical region than in a narrow and long region.

When dye molecules are injected into biological tissues, they are affected by the host tissue through binding or collisions. Therefore, the emission spectra of the dye may have different spectral peak positions in different tissues, such as diseased vs. normal tissues. Because the spectral peaks of laser action are much sharper than those of fluorescence, the laser action should be more sensitive in detecting any shift of the spectral peak than fluorescence. This unique feature may find applications in diagnosis of superficial lesions. Comparison of laser-action spectra between normal and abnormal tissues will test this hypothesis.

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Summary

Laser action in dye-infused biological tissues was observed and was speculated to be caused by scattering enhanced amplification of spontaneous emission. The sharp spectral peaks of laser action may carry information of the host tissue that can be used for high-sensitivity diagnosis of disease.

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